

Theranostic ultrasound-mediated blood-brain barrier opening and viral delivery with a novel pulse sequence

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Objectives:

This study aims to characterize the gene delivery potential of theranostic ultrasound-mediated blood-brain barrier opening (BBBO) in mice using a novel bilateral sonication pulse sequence.

Methods:

A P4-1 (Philips) phased array (1.5 MHz Tx frequency, 1.0 MPa derated peak-negative pressure, $\pm 3.72^\circ$ electronic steering angles), was operated by a custom Verasonics MATLAB script to employ the rapid alternating steering angles (RASTA) pulse sequence for bilateral viral delivery (AAV9-CAG-GFP) at two systemic doses: 6.5×10^{10} gc/mouse and 1.0×10^{10} gc/mouse. RASTA consists of interleaved focused transmits deployed on the right and left hemispheres with hemisphere-specific short pulse lengths (1.5, 5, and 10 cycles).

Results:

GFP reporter transgene expression was detectable with fluorescence microscopy on coronally-oriented brain sections 4 weeks after BBBO with TUS RASTA at both AAV9 dose groups, with increasing GFP expression observed with pulse length (Fig. 1A-I). 1.5, 5 and 10-cycle pulse lengths elicited GFP reporter coverage dependent on pulse length ranging from 0.042 mm^3 - 0.367 mm^3 at a dose of 6.5×10^{10} gc/mouse (Fig. 1D-F, K), and 0.004 mm^3 - 0.014 mm^3 at a dose of 1.0×10^{10} gc/mouse (Fig. 1G-I, L). A 6.5-fold increase in AAV dose yielded an average 7.5-fold increase in GFP reporter gene expression across all pulse lengths evaluated (Fig. 1J).

Conclusions:

TUS RASTA in conjunction with systemically administered AAV9 presented a novel bilateral gene delivery platform within a single diagnostic imaging array configuration. Such a platform could provide a computationally flexible alternative to existing preclinical ultrasound-guided focused ultrasound (USgFUS) systems currently employed for viral delivery to the brain.

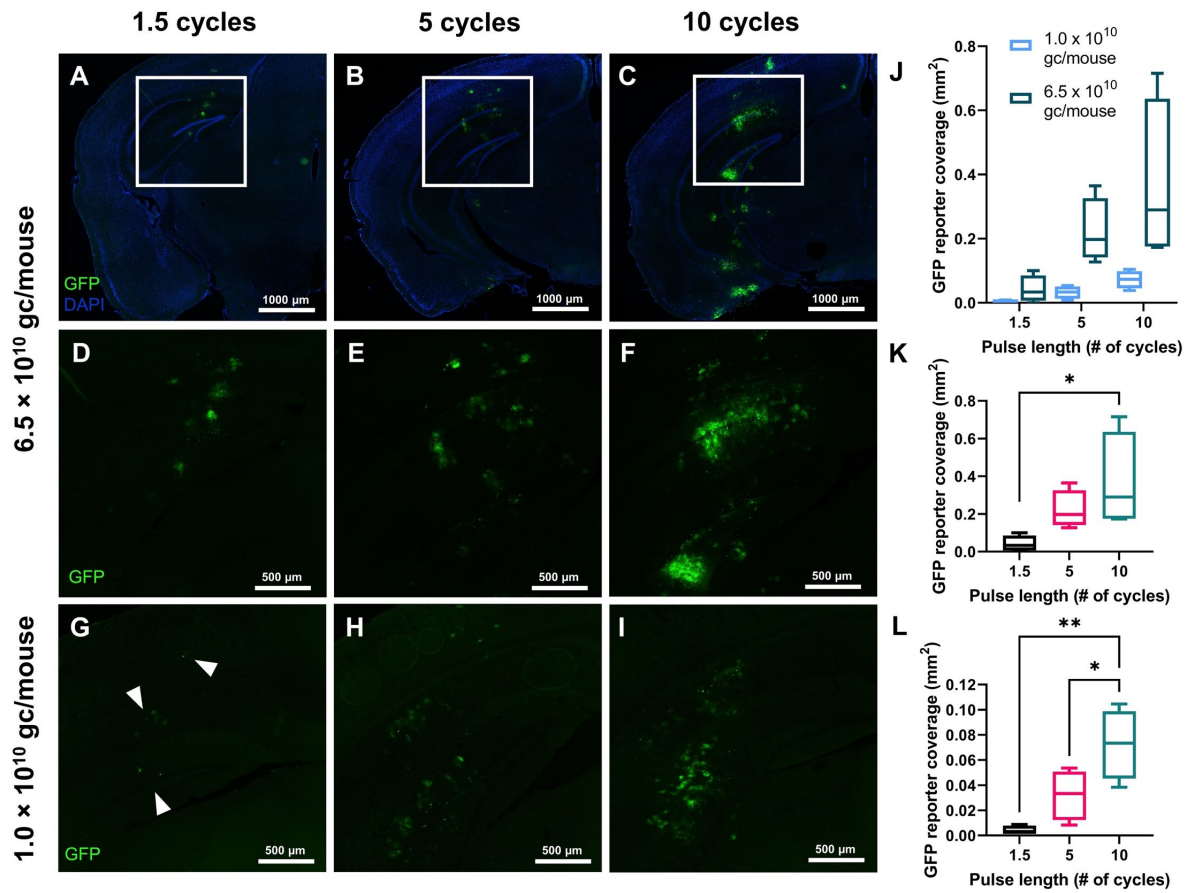


Figure 1: GFP expression at a dose of **A-F)** 6.5×10^{10} gc/mouse and **G-I)** 1.0×10^{10} gc/mouse. Quantification of GFP transgene coverage at a dose of **J-K)** 6.5×10^{10} gc/mouse and **J,L)** 1.0×10^{10} gc/mouse. White boxes in (A-C) denote the approximate anatomical regions of enlarged images shown in (D-I). * $p < 0.05$, ** $p < 0.01$, one-way ANOVA with Tukey's multiple comparisons test.